OBSERVATIONS ON THE INFLUENCE OF INORGANIC IRON ON THE VIRULENCE OF P. PESTIS CULTIVATED ON ARTIFICIAL NUTRIENT MEDIA

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OBSERVATIONS ON THE INFLUENCE OF INORGANIC IRON ON THE VIRULENCE OF P. PESTIS CULTIVATED ON ARTIFICIAL NUTRIENT MEDIA

[Following is the translation of an article by L. N. Klassovskiy and L. I. Terentyeva, published in the Russian-language periodical Materialy Nauchnoy Konferentsii po Prirodnoy Ochagovosti i Profilaktike Chumy (Materials from the Scientific Conference on the Natural Focalness and Prophylaxis of Plague), Alma-Ata, Feb., 1963, pages 110-112. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

Frequent reseedings of virulent cultures of plague bacteria on artificial nutrient media regularly lead to the attenuation of virulent properties (Korobkova, 1965; Yelfimova and Khakina, 1956; Klassovskiy and Osadchaya, 1959). The intensity of the process of lowering of virulence depends to a specific degree on the chemical composition of the nutrient media (Wessman, Miller, and Surgalla, 1958; Ivanov, 1959; Delwich, Fukui et al, 1959).

Below the results are presented of the experiments on the study of the influence of ferrous sulfate on the rate of loss of virulence by the plague causative agent during frequent reseedings on Hottinger agar. In beginning this investigation, we took into consideration the facts which are available in the literature concerning the significance of inorganic iron for the development of pathogenic properties in the plague microbe (Jackson and Burrows), 1956; Avanyan and Gubina, 1960).

In the test we used a typical highly virulent glycerol-positive strain of the plague microbe 1435, isolated four years prior to the experiment from a gray marmot in Tyan-Shan.

The selection of this strain was conditioned, on the one hand, by the high virulence of the initial culture (subcutaneous administration of 10 microbial bodies was lethal for white mice), and on the other hand, by the ease with which the process of emergence and accumulation of avirulent forms set in in the population of this strain during cultivation on nutrient media.

In a preliminary test 20 subcultivations of this strain on Hottinger agar led to the loss of virulence.

Ferrous sulfate ($FeSO_4 \cdot 7H_2O$) was added to Hottinger agar in a

proportion of 50 mg per 100 ml of medium. Preliminarily a commercial preparation of iron was purified by repeated precipitation from an aqueous solution by ethyl alcohol with the drying of the precipitate on filter paper at room temperature. The test strain was subcultivated three times a week on agar with the addition (test) and without the addition (control) of the iron preparation. The incubation temperature was 28°C. Beginning with the second passage and then after every four subinoculations the cultures were tested for their virulence for white mice. Each time for testing virulence, 11 subcultures were taken: The general mass of the population and 10 subcultures, obtained by the selection of isolated colonies. The animals were infected subcutaneously with two-day cultures (dose of 100 thousand microbial bod'es) of the test variants, incubated on agar without the addition of iron. The latter made it possible to avoid inequality of conditions in the preparation of subcultures for inoculation.

Both in the test and in the control the presence of avirulent cells (colonies) in the cultures being passaged was established after 12 passages.

However, subsequently on the medium without iron (the control) the process of accumulation of avirulent specimens proceeded more intensively than in the test. Already with the 17th passage virulent colonies were not detected in the control population, and after 22 subcultivations the population as a whole ceased to kill white mice.

The process of losing virulence on the medium with iron proceeded significantly slower. Even after 52 subcultivations the overall mass of the population still continued to kill the animals, and individual subcultures from isolated colonies preserved their virulence following 42 subcultures.

In order to be convinced of the reliability of the results obtained, two analogous tests based on a simplified technique were carried out with strain 1435. The frequency of subcultures and the media were the same as in the previous test, only the virulence of the cultures was determined one time (in one test following 21 subcultures, and in another test -- following 33).

In the first additional test, following 21 subcultivations on agar without the addition of iron all ten tested colonies of strain 1435 turned out to be avirulent, though the population as a whole continued to kill some of the animals. As regards the cultures which were subcultured on a medium with the addition of a preparation of iron (50 mg per 100 ml of medium), not only the population as a whole but seven out of the 10 tested colonies turned out to be virulent.

In the second additional test the content of iron in the agar was doubled (100 mg per 100 ml of medium). The test for virulence, conducted after 33 subcultivations, showed that on ordinary Hottinger agar both the population as a whole and the 10 tested colonies became avirulent. On agar with the addition of the iron preparation, out of 10 colonies five continued to kill the animals with the typical symptoms of plague, and the population as a whole preserved virulence. Here it must be noted that the concentration of iron used in the second test exerted a noticeable depressing effect on the growth of the culture of causative agent.

In this manner all three tests produced corresponding results. This made a basis to conclude that the addition of ferrous sulfate to solid nutrient medium exerts a retarding influence on the process of the lowering of virulence of the plague microbe.

The mechanism of this phenomenon still remains unclear. It may be postulated that here an active role is played by the redox ferments of the hemin series, the most important component of which is iron.